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Date: August 21, 2001 By: Lynnea B Anderson

DOCKET NO.: 5525-0044.10

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF: Sydney Brenner	EXAMINER: not yet assigned
SERIAL NO.: not yet assigned	ART UNIT: not yet assigned
FILED: 20 August 2001	
FOR: **POLYMORPHIC DNA FRAGMENTS AND USES THEREOF**	

Preliminary Amendment

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to examination of the above application, please amend the application as follows:

In the Specification:

At page 1, line 1 of the specification, preceding "FIELD OF THE INVENTION", please insert the following paragraph:

This application is a U.S. National Stage filing under 35 U.S.C. §371 of PCT Appn. No. PCT/US00/04349, which claims priority to U.S. Provisional Appn. No. 60/121,023, filed February 22, 1999, and to U.S. Provisional Appn. No. 60/158,483, filed October 8, 1999, all of which are hereby incorporated by reference in their entirety.

In the Claims:

Please replace claims 1-12, 15-16 and 18 with the rewritten claims below, and add claims 19-20, as follows. Also enclosed, starting on a separate page following this response, is a marked copy of the presently amended claims showing all changes relative to the previous version.

1. A nucleic acid reference library derived from pooled DNA from at least two sources, said library comprising a heterogeneous mixture of nucleic acid fragments, wherein

each said fragment (a) is a portion of a polymorphic subregion of a polymorphic consensus sequence derived from said pooled DNA or (b) is derived from a non-polymorphic subregion,

each of said polymorphic subregions is bounded by first restriction sites and comprises an internal polymorphic restriction site which is different from said first site;

said polymorphic consensus sequence is the theoretical sequence obtained by (i) aligning said pooled DNA to provide maximum homology, and (ii) projecting each of said restriction sites onto said sequence; and

said library is enriched for fragments of type (a) relative to type (b).

2. A nucleic acid reference library according to claim 1, wherein at least a subpopulation of said nucleic acid fragments further comprise oligonucleotide tags, and different nucleic acid fragments are linked to different oligonucleotide tags.

3. The nucleic acid reference library according to claim 2, wherein said fragments are contained within a replicable vector.

4. The nucleic acid reference library according to claim 2, wherein said oligonucleotide tags comprise oligonucleotides of the form:

$$S_1S_2S_3 \ldots S_n$$

wherein each of S_1 through S_n are subunits consisting of an oligonucleotide having a length from 3 to 9 nucleotides and are selected from a minimally cross-hybridizing set, n is in the range of from 4 to 10, and said tag has a length in the range of from 12 to 60 nucleotides or base pairs.

5. A composition comprising subpopulations of microparticles, wherein each subpopulation comprises at least one microparticle, said microparticle comprising a polymorphic probe, wherein the polymorphic probe of each subpopulation is different from those of the other subpopulations, and comprises a portion of a polymorphic subregion of a polymorphic consensus

sequence as recited in claim 1.

6. The composition of claim 5, wherein each of said subpopulations further comprises a oligonucleotide tag, and different subpopulations comprise different tags.

7. The composition according to claim 6, wherein each said oligonucleotide tag is positioned between said microparticle and said polymorphic probe.

8. The composition according to claim 6, wherein each said oligonucleotide tags comprises an oligonucleotide of the form:

$$S_1S_2S_3 \ldots S_n$$

wherein each of S_1 through S_n are subunits consisting of an oligonucleotide having a length from 3 to 9 nucleotides and are selected from a minimally cross-hybridizing set, n is in the range of from 4 to 10, and said tag has a length in the range of from 12 to 60 nucleotides or base pairs.

9. An array comprising a solid support having defined regions on the surface thereof, wherein each region comprises a different polymorphic probe, and wherein each of said polymorphic probes comprises a portion of a polymorphic subregion of a polymorphic consensus sequence as recited in claim 1.

10. The array of claim 9, wherein each of said regions further comprises an oligonucleotide tag, and different subpopulations comprise different tags.

11. The array of claim 10, wherein each said oligonucleotide tag is positioned between said surface and said polymorphic probe.

12. The array according to claim 11, wherein each said oligonucleotide tag comprises an oligonucleotide of the form:

$$S_1S_2S_3 \ldots S_n$$

wherein each of S_1 through S_n are subunits consisting of an oligonucleotide having a length from

3 to 9 nucleotides and are selected from a minimally cross-hybridizing set, n is in the range of from 4 to 10, and said tag has a length in the range of from 12 to 60 nucleotides or base pairs.

13. A nucleic acid reference library of genomic DNA from a plurality of individuals comprising:

a heterogeneous mixture of restriction fragments of a first restriction endonuclease;

wherein said restriction fragments from the same locus contain members having one or more restriction site polymorphisms with respect to a second restriction endonuclease, the number of such members forming a proper subset of the total number of restriction fragments from said locus.

14. A nucleic acid reference library of genomic DNA from a plurality of individuals comprising:

a heterogeneous mixture of restriction fragments of a first restriction endonuclease;

wherein said restriction fragments from the same locus contain at least one member having one or more restriction site polymorphisms with respect to a second restriction endonuclease and at least one member without said restriction site polymorphism.

15. A method of making a reference library comprising a mixture of heterogeneous nucleic acid fragments, comprising:

digesting pooled nucleic acid comprising first restriction sites with a first restriction endonuclease to produce a mixture of restriction fragments;

forming a first population of single stranded nucleic DNA fragments from a first subpopulation of said restriction fragments, wherein said first subpopulation of restriction fragments comprises a second restriction site which is different from said first restriction site;

forming a second population of single stranded DNA fragments from a second subpopulation of said restriction fragments, wherein said second subpopulation of said restriction fragments do not contain said second restriction site,

and wherein said first single stranded DNA fragments are complementary to said second single stranded DNA fragments when said single stranded DNA fragments are derived from the same restriction fragment;

hybridizing the first and second populations of single stranded DNA fragments to form a

population of duplexes; and

isolating said duplexes to form a reference population of restriction fragments.

16. The method of Claim 15, further comprising the step of pretreating said pooled nucleic acid to enrich for non-repetitive sequences.

17. A method for determining the ratio of a polymorphic subregion between at least two different pools of test nucleic acid, comprising

generating a first pool of restriction endonuclease fragments from a first pool of test nucleic acids comprising first restriction sites, by digesting said pool with a first restriction endonuclease;

generating a second pool of restriction endonuclease fragments from a second pool of test nucleic acids comprising first restriction sites, by digesting said pool with said first restriction endonuclease;

enriching said first and said second pools of restriction fragments for those fragments which contain a polymorphism associated with a second restriction site, to form first and second enriched populations;

contacting said first and said second enriched populations with a reference library comprising probes enriched for subregions which are polymorphic for said second restriction site; and

determining the ratio of binding of said probes with said first and said second enriched populations.

18. The method of claim 17, wherein said first pool of test nucleic acids is from a population of individuals having a first phenotype and said second pool of test nucleic acids is from a population of individuals having a second phenotype.

19. (New) The method of claim 17, wherein said enriching comprises selecting fragments from said pools which lack said second restriction site, and said contacting comprises contacting said selected fragments with probes which contain said second restriction site.

20. (New) The method of claim 17, wherein said enriching comprises selecting fragments from said pools which contain said second restriction site, and said contacting comprises contacting said selected fragments with probes which lack said second restriction site.

Remarks

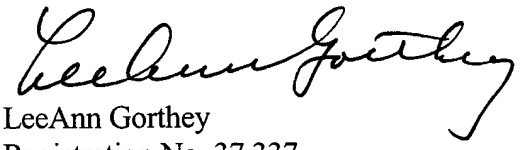
Several of the claims have been amended to clarify language. Support for the amendments to claim 1 is found in the specification at, for example, the following locations: for "derived from pooled DNA from at least two sources", at page 6, line 24; for the description of the polymorphic consensus sequence, page 6, line 24 to page 7, line 1 and page 7, lines 15-17; for non-polymorphic subregions, at page 8, lines 10-12, and for enrichment of "type (a)" (i.e. portions of polymorphic subregions) relative to "type (b)" (i.e. derived from non-polymorphic subregions), at page 7, lines 26-29 and page 8, lines 7-12.

Support for added dependent claims 19 and 20 is found, respectively, at page 25, lines 4-8, and at the paragraph bridging pages 25-26.

Entry of this amendment prior to examination is respectfully requested. No new matter is added by any of the amendments.

No further fees are believed necessary with this communication. However, the Commissioner is hereby authorized and requested to charge any deficiency in fees herein, or credit any overpayment, to Deposit Account No. 50-0665.

Respectfully submitted,



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